

POSTER SESSION 1

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Group 3 - Signalling - Growth factors & Mechanics

P90

Necrotic cardiomyocytes release soluble pro-inflammatory molecule(s) inducing il1r/myd88-dependent inflammatory responses in cardiac fibroblasts

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Background: Inflammation comes out to be a critical biological process in the pathophysiology of myocardial infarction (MI). We hypothesize that this inflammation is triggered by necrotic cardiomyocytes (Cmc) that release a set of endogenous molecules (DAMPs: danger-associated molecular patterns) activating inflammatory responses in cardiac fibroblasts.

Aim: Analyze in vitro the immune activation of cardiac fibroblasts exposed to necrotic Cmc conditioned media.

Methods: Primary neonatal murine cardiac fibroblasts and Cmc were obtained by digestion of neonatal hearts and differential plating technique allowing a selection for cardiomyocytes and cardiac

fibroblasts. Cmc were killed by necrotic stimuli including oxidants (hydrogen peroxide) and mechanic stresses (freeze-thaw). Necrosis was assessed using Hoechst/PI stainings. Fibroblasts were exposed to necrotic Cmc conditioned media and mRNA expression of inflammatory genes was measured by real-time PCR and ELISA. Activation of signaling pathways was analyzed by western blot. We used cardiac cells from Myd88^{-/-}, Trif^{-/-} and Nlrp3^{-/-} animals to evaluate the contribution of TLRs/IL-1R and NLRP3 inflammasome in the sensing of necrotic DAMPs.

Results: mRNA expression of chemokines such as MCP-1, MIP-2 and IP-10 were induced in fibroblasts exposed to necrotic Cmc conditioned media. Alternatively, fibroblasts exposed to necrotic fibroblasts conditioned media showed a lower increase in mRNA expression of these chemokines. In addition, in fibroblasts from Myd88^{-/-} mice, response to Cmc conditioned media was fully abrogated whereas no difference was observed in Trif^{-/-} and Nlrp3^{-/-} fibroblasts.

Conclusion: Cardiac fibroblasts are able to produce a rapid and specific inflammatory response to necrotic Cmc conditioned media involving the expression of neutrophil and monocyte chemoattractants. The dependence on MyD88 adaptor protein strongly suggests that this response relies on TLR/IL-1R signaling. These results engage cardiac fibroblasts as key players in post-MI inflammatory responses as they are able to sense DAMPs from necrotic Cmc and possibly recruit inflammatory cells. Research supported by the Swiss National Science Foundation, Grant n° 310030_135394/1